Amendments to the Specification:

- In the section entitled "Brief Description of the Drawings", which was inserted by amendment at page 11 of the specification, immediately preceding line 6, in the response filed October 19, 2004, please amend the paragraph starting with "Figure 4 illustrates" as follows:

Figure 4 illustrates discrimination between colonies originated from two different templates. Figure 4a shows the images of colonies made from both templates and negative controls; Figure 4b shows the colonies from both templates at the same position in the same well visualised with two different colours and negative controls; Figure 4c shows the coordinates of both colony types in a sub-section of a microscopy field[[;]] and ~~Figure 4d~~ demonstrates that colonies from different templates do not coincide.

- In the section entitled "Brief Description of the Drawings", which was inserted by amendment at page 11 of the specification, immediately preceding line 6, in the response filed October 19, 2004, please amend the paragraph starting with "Figure 8 shows" as follows:

Figure 8 shows hybridization of probes to oligonucleotides attached to Nucleolink, before and after PCR cycling. The figure shows R58 hybridization to CP2 (5'-(phosphate)-TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), closed circles; CP8 (5'(amino-hexamethylene)- TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), closed triangles; CP9 (5'(hydroxyl)-TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), diamonds; CP10 (5'(dimethoxytrityl)-TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), open circles; and CP11 (5'(biotin)-TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), open triangles.

- Please amend the paragraph starting at page 13, line 13 as follows:

"Colony primer" as used herein refers to an entity which comprises an oligonucleotide sequence which is capable of hybridizing to a complementary sequence and initiate a specific polymerase reaction. The sequence comprising the colony primer is chosen such that it has maximal hybridising activity with its complementary sequence and very low non-specific hybridising activity to any other sequence. The sequence to be used as a colony primer can include any sequence, but preferably includes 5'-AGAAGGAGAAGGAAAGGGAAAGGG (SEQ ID NO: 1) or 5'-CACCAACCCAAACCAACCCAAACC (SEQ ID NO: 2). The colony primer can be 5 to 100 bases in length, but preferably 15 to 25 bases in length. Naturally occurring or non-naturally occurring nucleotides may be present in the primer. One or two different colony primers may be used to generate nucleic acid colonies in the methods of the present invention.

- Please amend the paragraph starting at page 44, line 28 as follows:

Figure 8[[:]] shows hybridization of probes to oligonucleotides attached to Nucleolink, before and after PCR cycling. The figure shows R58 hybridization to CP2 (5'-(phosphate)-TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), closed circles, CP8 (5'(amino-hexamethylene)- TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), closed triangles, CP9 (5'(hydroxyl)-TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), diamonds, CP10 (5'(dimethoxytrityl)-TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), open circles; and CP11 (5'(biotin)-TTTTTTTTTT AGAAGGAGAA GGAAAGGGAA AGGG) (SEQ ID NO: 6), open triangles.

- Please amend the paragraph starting at page 45, line 16 as follows:

The properties of the colony primers have been chosen based on a selection for oligonucleotide primers that show little non-specific nucleotide incorporation in the presence of heat-stable DNA polymerases. The colony primers, CPα

(5'-pCACCAACCCAAACCAACCCAAACC) (SEQ ID NO: 2) and CPβ (5'-pAGAAGGAGAAGGAAAGGGAAAGGG) (SEQ ID NO: 1) have been selected due to their low incorporation of radiolabeled ~~[α-^{32}P-dCTP]~~ α^{32}P-dCTP in the presence of a stable DNA polymerase (AmpliTaq, Perkin Elmer, Foster City, CA) in the standard buffer and under thermocycling conditions (94°C for 30 seconds, 65°C for 1 minute, 72°C for 2 minutes, 50 cycles).

- Please replace the Table starting on page 48, line 5 (Table 1) with the following:

Name	SEQ ID NO:	DNA sequence	Coordinates (orientation)	Oligonucleotide Modification	Use
TP1	3	GAGGCCAGAACAGT TCAAGG	9810 (R)		Template 3.2 Kb
TP2	4	CCTGTGACAAGACG ACTGAA	6550 (F)		Template 3.2 Kb
CP1	5	TTTTTTTTTTCACC AACCCAAACCAACC CAAACC	None	5'P	Generate colonies
CP2	6	TTTTTTTTTTAGAA GGAGAAGGAAAGGG AAAGGG	None	5'P	Generate colonies
CP3	7	TTTTTTTTTTCACC AACCCAAACCAACC CAAACC	None	5'SH	Generate colonies
CP4	8	TTTTTTTTTTAGAA GGAGAAGGAAAGGG AAAGGG	None	5'SH	Generate colonies
CP5	9	AGAAGGAGAAGGAA AGGGAAAGGGTTTT TTTTTTTTTTTNN	None	5'P	Generate colonies
CP6	10	AGAAGGAGAAGGAA AGGGAAAGGGGG	None	5'P	Generate colonies
CP7	5	TTTTTTTTTTCACC AACCCAAACCAACC CAAACC	None	5'(NH2)	Generate colonies
CP8	6	TTTTTTTTTTAGAA GGAGAAGGAAAGGG AAAGGG	None	5'(NH2)	Generate colonies
CP9	6	TTTTTTTTTTAGAA GGAGAAGGAAAGGG AAAGGG	None	5'(OH)	Control oligo
CP10	6	TTTTTTTTTTAGAA GGAGAAGGAAAGGG AAAGGG	None	5'(DMT)	Control oligo

CP11	6	TTTTTTTTTTAGAA GGAGAAGGAAAGGG AAAGGG	None	5'(biotin)	Control oligo
TPA1	12	AGAAGGAGAAGGAA AGGGAAAGGGCCTG TGACAAGACGACTG AA	6550 (F)	5'P	Template A
TPA2	13	TTTTTTTTTTAGAA GGAGAAGGAAAGGG AAAGGGGCGGCCGC TGAGGCCAGTGGAA GTCAGA	7403 (R)	5'P	Template A
TPB3	14	TTTTTTTTTTCACC AACCCAAACCAACC CAAACCGAGCTCAG GCTGAGGCAGGAGA ATTG	9049 (F)	None	Template B'
TPB1	15	AGAAGGAGAAGGAA AGGGAAAGGGGAGC TGAGGAGGAAGAGA GG	9265 (F)	None	Template B
TPB2	16	AGAAGGAGAAGGAA AGGGAAAGGGGCGG CCGCTCGCCTGGTT CTGGAAGACA	8411 (R)	5'P	Template B
TPB4	16	AGAAGGAGAAGGAA AGGGAAAGGGGCGG CCGCTCGCCTGGTT CTGGAAGACA	9265 (R)	5'SH	Template B'

- Please amend the paragraph starting at page 49, line 15 as follows:

A 3.2 Kb DNA fragment was taken as a model system to demonstrate the feasibility of colony generation from random primer PCR amplification. This strategy can be applied to sequencing of DNA fragments of approximately 100 Kb in length and, by combination of fragments, to whole genomes. A fragment of DNA of 3.2 Kb was generated by PCR from human genomic DNA using PCR primers[[;]] TP1 5'-pGAGGCCAGAACAGTTCAAGG (SEQ ID NO: 3) and TP2 5'-pCCTGTGACAAGACGACTGAA (SEQ ID NO: 4), as described in example 1. The 3.2 Kb fragment was cut in smaller fragments by a combination of restriction enzymes (EcoR1 and HhaI, yielding 4 fragments of roughly 800 bp). The cut or uncut fragment DNAs were then mixed with the degenerate primer, P252 (5'-pTTTTTTTTTTISISISISISIS (SEQ ID NO: 17), where I stands for inosine (which pairs

with A, T and C) and S stands for G or C) and covalently coupled to the Nucleolink wells (Nunc, Denmark). The tubes were then subjected to random solid phase PCR amplification and visualized by hybridisation with labeled DNA probes, as will be described in Example 2a.

- Please amend the paragraph starting at page 50, line 17 as follows:

A colony primer (CP2, 5'-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) (SEQ ID NO: 6), phosphorylated at its 5' terminus (Microsynth GmBH, Switzerland), was attached onto Nucleolink plastic microtitre wells (Nunc, Denmark) in the presence of varying doses of Template A (prepared as described in example 1). [[8]] Eight wells were set up in duplicate with seven 1/10 dilutions of template with CP2, starting with the highest concentration of 1 nM.

- Please amend the paragraph starting at page 51, line 25 as follows:

Probe: The probe was a DNA fragment of 1405 base pairs comprising the sequence of the template at ~~their~~ its 3' end (nucleotide positions 8405 to 9259). The DNA probe was synthesized by PCR using two primers: p47 (5'- GGCTAGGAGCTGAGGAGGAA) (SEQ ID NO: 20), amplifying from base 8405, and TP2, biotinylated at 5' end, amplifying from base 9876 of the antisense strand.

- Please amend the paragraph starting at page 54, line 21 as follows:

A colony primer (CP2: 5'pTTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) (SEQ ID NO: 6), phosphorylated at its 5' terminus ~~termini~~ (Microsynth GmbH, Switzerland), was grafted onto Nucleolink plastic microtitre wells (Nunc, Denmark) in the presence of varying doses of the two templates A and B (prepared as described in example 1). Series of 8 wells were set up in triplicate with seven 1/10 dilutions of both templates starting with the highest concentration of 1 nM. Template dilutions are set up in opposite directions such that the

highest concentration of one template coincides with the lowest of the other.

- Please amend the paragraph starting at page 67, line 5 as follows:

Colony primers CP1 (5'-pTTTTTTTTTTCACCAACCCAAACCAACCCAAACC) (SEQ ID NO: 5) and CP2 (5'-pTTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) (SEQ ID NO: 6) which are 5' phosphorylated (Microsynth GmbH, Switzerland) and DNA template B (prepared as described in example 1) were 5' covalently attached onto 5 mm diameter glass slides (Verrerie de Carouge, Switzerland) to [[a]] final concentrations of 1 pM and 10 nM, respectively, as follows: 2 nmoles of each primer were added to 0.2 nmoles of template in 1 ml of solution A (41 µl of Methylimidazole (Sigma, #M-8878) in 50 ml H20, pH adjusted to 7 with HCl) and then mixed 1:1 with solution D (0.2 mM EDC in 10 ml of solution A). On both glass slides sides, 3.5 µl of the mixture were loaded, and incubated over night at room temperature. The glass slides were then briefly rinsed with 5xSSC buffer and placed at 100°C in 10mM Tris buffer pH 8.0 for 2x5'.

- Please amend the paragraph starting at page 67, line 28 as follows:

Glass slides were then individually placed onto a Microamp™ reaction tube (Perkin Elmer) containing 170 µl of PCR mix, and DNA colonies were then generated using Taq polymerase (AmpliTaq, PE-Applied Biosystems Inc., Foster City CA) with 50 cycles (94C/60", 60C/3', 72C/6') in a MTC 200 thermo-cycler (MJ Research, Watertown, MA). Each slide was digested twice using 1.3 units of Pvu II (Stratagene) in NEB 2 buffer (New England Biolabs) for 45 minutes at 37°C. After digestion, the tubes were placed at 100°C in 10mM Tris buffer pH 8.0 for 2x5', then blocked with filtered (Millex GV4, Millipore) 1 mg/ml BSA in 2xSSC buffer for 30' at room temperature and rinsed first in 2xSSC 0.1% SDS buffer then in 5xSSC buffer. Each slide was incubated over night at room temperature with a 5xSSC/0.1% Tween 20 buffer containing 1 µM of the sequencing primer p181 (CGACAGCCGGAAGGAAGAGGGAGC) (SEQ ID NO: 18) overnight at room temperature. Controls without primer were kept in 5xSSC 0.1% Tween 20 buffer. Glass

slides were washed 2 times in 5xSSC 0.1% SDS at 37C for 5' and rinsed in 5xSSC. Primer p181 can hybridize to template BI and the sequence following p181 is CAGCT.... In order to facilitate focusing, green fluorescent beads have been adsorbed to the bottom of the well by incubating each well with 20 µl of a 1/2000 dilution of 200 nm yellow/green fluorescent, streptavidin coated FluoSpheres(R) (Molecular Probes, Eugene, OR) in 5X SSC for 20" at room temperature.

- Please amend the paragraph starting at page 71, line 19 as follows:

CDNA synthesis - Synthetic mRNA was mixed with mouse liver poly A+ mRNA at different molar ratios (1:1, 1:10, 1:100) and cDNA synthesis on the mixture of synthetic and mouse liver mRNA was performed using the "SMART PCR cDNA synthesis kit" (Clontech, Palo Alto CA) with some minor modifications. In a cDNA reaction, approximately 1 µg of the mRNA mixture was mixed with the -primer CP5, having at the 5'-end the sequence of CPβ, (5'p-AGAAGGAGAAGGAAAGGGAAAGGGTTTTTTTTTTTTTTTNN) (SEQ ID NO: 9). This primer has been used to make the 1st strand cDNA synthesis. For the 2nd strand synthesis, the "SMART" technique has been used. The basis of the SMART synthesis is the property of the Moloney murine viral reverse transcriptase to add three to five deoxycytosine residues at the 3'-termini of first strand cDNA, when the mRNA contains a 5'-methylguanosine-cap (SMART user manual, Clontech, Palo Alto CA). A CP6 primer, which contains the sequence of CPP plus AAAGGGGG at the 31 end, (5'p-AGAAGGAGAAGGAAAGGGAAAGGGGG) (SEQ ID NO: 10), has been used for the 2nd strand cDNA synthesis. Buffer and SUPERSCRIPT™ II RNase H-reverse transcriptase from Moloney murine leukemia virus (Life Technologies, Ltd.) were used as described in the instructions and the reaction was carried out at 42°C for 1 hr. The cDNA was assayed by PCR using the primer p251, which contains a fragment of the CPβ sequence, (5'-GAGAAGGAAAGGGAAAGG) (SEQ ID NO: 19), with Taq DNA polymerase (Platinum Taq, Life Technologies, Ltd.).

- Please amend the paragraph starting at page 72, line 10 as follows:

Preparation of DNA colonies - The 5'p-cDNA was mixed with different concentrations of the solid phase colony primer, CP2 (5'p-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) (SEQ ID NO: 6) and chemically bound to Nucleolink PCR tubes (NUNC) following manufacturer instructions. DNA colonies were then generated using Taq polymerase (AmpliTaq Gold, PE-Applied Biosystems Inc., Foster City CA) with 30 cycles (94C/30", 65C/1', 72C/1.5') in a MTC 200 thermo-cycler (MJ Research, Watertown, MA).

- Please amend the paragraph starting at page 74, line 16 as follows:

Oligonucleotide primers were attached onto Nucleolink plastic microtitre wells (Nunc, Denmark) in order to determine optimal coupling times and chemistries. Oligonucleotides; CP2 (5'-(phosphate)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG), CP8 (5'-(amino-hexamethylene)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG), CP9 (5'(hydroxyl)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG), CP10 (5'-(dimethoxytrityl)- TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) and CP11 (5'(biotin)-TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG) (all SEQ ID NO: 6), (Microsynth GmbH, Switzerland), were attached to Nucleolink microtitre wells as follows (8 wells each); to each well 20 µl of a solution containing 0.1 µM oligonucleotide, 10mM 1-methyl-imidazole (pH 7.0) (Sigma Chemicals) and 10mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (pH 7.0) (Sigma Chemicals) in 10mM 1-methyl-imidazole. The wells were then sealed and incubated at 50°C for varying amounts of time. The coupling reaction was terminated at specific times by rinsing twice with 200 µl of RS (0.4 N NaOH, 0.25% Tween) and twice with 200 µl TNT (100 mM Tris HCl pH 7.5, 150 mM NaCl, 0.1% Tween 20). Tubes were dried at 50°C for 30' and were stored in a sealed plastic bag at 4°C.

- Please amend the paragraph starting at page 75, line 6 as follows:

Stability was tested under colony growing conditions by adding a PCR mix (20 µl of four dNTPs (0.2 mM), 0.1% BSA, 0.1% Tween 20, 8% DMSO (dimethylsulfoxide, Fluka, Switzerland), IX PCR buffer). The wells were then placed in the thermocycler and for 33 repetitions under the following conditions: 94°C for 45 seconds, 60°C for 4 minutes, 72°C for 4 minutes. After completion of this program, the wells were rinsed with 5xSSC, 0.1% Tween 20 and kept at 8°C until further use. Prior to hybridization wells are filled with 50 µl 5xSSC, 0.1% Tween 20 heated at 94°C for 5 minutes and stored at RT. Probe: Oligonucleotide probes, R57 (5'(phosphate)-GTTTGGGTTGGTTTGGGTTGGTG, control probe) (complementary to SEQ ID NO: 2) and R58 (5'-(phosphate)-CCCTTTCCCTTTCCTTCTCCTTCT (complement of SEQ ID NO: 1), which is complementary to CP2, CP8, CP9, CP10 and CP11) were enzymatically labeled at their 5' end terminus with ~~[γ-^{32}P]~~ γ-^{32}P dATP (Amersham, UK) using the bacteriophage T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Excess ^{32}P dATP was removed with a Chroma Spin column TE-10 (Clontech, Palo Alto CA). Radiolabeled oligonucleotides (0.5 µM in 5xSSC, 0.1% Tween 20) were then hybridized to the oligonucleotide derivatized Nucleolink wells at 37°C for two hours. The wells were washed 4 times with 5xSSC, 0.1% Tween 20 at room temperature, followed by a wash with 0.5xSSC, 0.1% Tween for 15' at 37°C. Wells were then assayed for bound probe by scintillation counting.

- Please insert the enclosed paper copy of the Sequence Listing, following the last page (page 76) of the specification, replacing any previous copy of the Sequence Listing.